



Resurrecting the genus *Geomorium*: Systematic study of fungi in the genera *Underwoodia* and *Gymnohydnotrya* (Pezizales) with the description of three new South American species

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Key words

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truffle systematics
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Abstract Molecular phylogenetic analyses have addressed the systematic position of several major Northern Hemisphere lineages of *Pezizales* but the taxa of the Southern Hemisphere remain understudied. This study focuses on the molecular systematics and taxonomy of Southern Hemisphere species currently treated in the genera *Underwoodia* and *Gymnohydnotrya*. Species in these genera have been identified as the monophyletic /*gymnohydnotrya* lineage, but no further research has been conducted to determine the evolutionary origin of this lineage or its relationship with other *Pezizales* lineages. Here, we present a phylogenetic study of fungal species previously described in *Underwoodia* and *Gymnohydnotrya*, with sampling of all but one described species. We revise the taxonomy of this lineage and describe three new species from the Patagonian region of South America. Our results show that none of these Southern Hemisphere species are closely related to *Underwoodia columnaris*, the type species of the genus *Underwoodia*. Accordingly, we recognize the genus *Geomorium* described by Spegazzini in 1922 for *G. fuegianum*. We propose the new family, *Geomoriaceae* fam. nov., to accommodate this phylogenetically and morphologically unique Southern Hemisphere lineage. Molecular dating estimated that *Geomoriaceae* started to diverge from its sister clade *Tuberaceae* c. 112 MYA, with a crown age for the family in the late Cretaceous (c. 67 MYA). This scenario fits well with a Gondwanan origin of the family before the split of Australia and South America from Antarctica during the Paleocene-Eocene boundary (c. 50 MYA).

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INTRODUCTION

The *Pezizales* is a diverse order of fungi that is globally distributed and contains species with a variety of trophic modes, including saprobes, pathogens and ectomycorrhizal (EcM) fungi (Hansen & Pfister 2006). Although molecular phylogenetic analyses have addressed the systematic position of several major groups, the taxa from the Southern Hemisphere remain understudied. Bonito et al. (2013) produced the first comprehensive molecular phylogeny for the family *Tuberaceae* (*Pezizales*). They documented significant *Tuberaceae* diversity in the Southern Hemisphere and determined that all of the known species in the family are EcM. They also discovered that species of truffles in the genus *Gymnohydnotrya* and the Southern Hemisphere *Underwoodia* species form a clade that is distinct from both *Tuberaceae* and *Helvellaceae*. This clade was referred to as the /*gymnohydnotrya* lineage (Bonito et al. 2013). This Southern Hemisphere clade was noted as the

/gymnohydnotrya lineage because it was unclear whether these fungi were related to the Northern Hemisphere *Underwoodia columnaris*, the type species of the genus *Underwoodia*.

The genus *Underwoodia* was first described by Peck (1890) based on the new species, *U. columnaris*, from upstate New York. *Underwoodia columnaris* has ornamented ascospores and epigeous ascomata that are elongated, hollow and columnar. *Underwoodia columnaris* has also been reported from the Northeastern and Midwestern USA and Canada, but is rarely collected (Nusslé 1936; MycoPortal 2018). This is the only described *Underwoodia* species found in the Northern Hemisphere.

Thirty-two years after Peck introduced the genus *Underwoodia*, Spegazzini (1922) described *Geomorium* as a new monospecific genus with *G. fuegianum* as the only species. This species was collected in *Nothofagaceae* forests of Tierra del Fuego. The morphological similarities between *U. columnaris* and *G. fuegianum* were considered significant by Gamundí (1957) who consequently transferred *G. fuegianum* to *Underwoodia* as *U. fuegiana*. Since the description of *U. fuegiana*, Gamundí & Horak (1979) added the South American species *Underwoodia singeri* and recognized two varieties within that species: *U. singeri* var. *singeri* and *U. singeri* var. *fulvostipitata*. The varieties were differentiated by hymenium colour and texture as well as ascospore size and ornamentation. *Underwoodia singeri* var. *fulvostipitata* was distinguished by a darker, more viscid hymenium and ascospores that are larger than the typical variety (Gamundí & Horak 1979). The Australasian species, *Underwoodia beatonii*, was later described by Rifai (1968) and is morphologically similar to *U. fuegiana*.

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Species in the genus *Underwoodia* have thus far been classified as members of the *Helvellaceae* (Seaver 1918, Gamundi 1957, Korf 1972). This family placement reflects a similarity in ascumatal form; all these species have a sterile stipe and columnar form with a sometimes convoluted, folded fertile hymenial region. Anatomically these *Underwoodia* species have excipular tissues that are similar to *Helvellaceae* species. The outer excipulum and stipe tissue is formed of barrel-shaped cells that are parallel to one another and perpendicular to the outer surface, forming a palisade-like layer. Furthermore, the ascospores are similar in their thick spore walls, the presence of a single, large guttule and isolated rounded warts on the surface. Although most authors have accepted the genus *Underwoodia* as a distinct entity (Korf 1972, Gamundi 2010), some authors such as Eckblad (1968) and Harmaja (1974) have considered species of *Underwoodia* within the genus *Helvella*. However, phylogenetic analyses that included *U. columnaris* and various *Underwoodia* from the Southern Hemisphere (e.g., O'Donnell et al. 1997, Bonito et al. 2013, Landeros et al. 2015) have suggested that *Underwoodia* is paraphyletic but that all species appear to be distinct from *Helvella*. Other species have been combined in *Underwoodia* but they either have not been widely recognized (e.g., *Underwoodia fuegiana* var. *cabrinii*) (Raithelhuber 1983) or were considered only distantly related (e.g., *Underwoodia campbellii* and *Underwoodia sparassoides*, both considered synonyms of *Peziza proteana*) (Korf 1956).

The /gymnohydnotrya lineage also includes truffle-like species in the genus *Gymnohydnotrya* for which the lineage was named. Species of *Gymnohydnotrya* are either hypogeous or subhypogeous and exothecial (the ascumata lack a peridium and have a convoluted external hymenial layer). *Gymnohydnotrya* species have highly ornamented ascospores with thick walls and asci that lack opercula. The genus *Gymnohydnotrya* was described as being similar to the genus *Hydnotrya* except that the ascumata of *Hydnotrya* species are ptychothecial (i.e., having a convoluted hymenium enclosed by a peridium) (Zhang & Minter 1989). Zhang & Minter (1989) included three species in *Gymnohydnotrya* which are endemic to Australia: *G. australiana*, *G. echinulata* and *G. ellipsospora*. Zhang & Minter (1989) designated *G. australiana* as the type species and placed the genus in *Helvellaceae*.

Recently, we discovered several specimens of *Underwoodia*-like and *Gymnohydnotrya*-like species in *Nothofagaceae*-dominated forests in South America. Morphological and molecular analyses indicated at least two novel species in the /gymnohydnotrya lineage among our collections. We also obtained fresh collections of the Northern Hemisphere species *Underwoodia columnaris*, which enabled us to successfully sequence DNA from this rarely collected species for additional phylogenetic markers. The discovery of these new specimens spurred new investigations into the systematics of the /gymnohydnotrya lineage.

The goals of this study are to:

1. describe the morphology of known and novel species in the /gymnohydnotrya lineage;
2. place these fungi within a multi-locus phylogenetic framework that includes species of *Underwoodia*, *Gymnohydnotrya*, *Helvellaceae*, *Morchellaceae*, *Discinaceae*, and *Tuberaceae*;
3. determine whether the genus *Underwoodia* is monophyletic by examining the phylogenetic relationships among *Underwoodia columnaris* and the Southern Hemisphere *Underwoodia* species; and
4. estimate lineage divergence times by calibrating our phylogeny using a molecular clock to determine the timing of diversification events in the *Tuberaceae*, *Helvellaceae*, and the /gymnohydnotrya lineage.

MATERIALS AND METHODS

Taxon sampling and specimens studied

Ascomata of gymnohydnotrya-like and underwoodia-like species were collected in Patagonia (Chile and Argentina) during several field trips from 2008–2017. Fungi were located by searching through leaf litter and soil with a garden cultivator rake. Samples were placed in plastic boxes and transported to the field laboratory within 8 h. Macroscopic photos of fresh specimens were taken in the laboratory. Fresh pieces were stored in CTAB solution to preserve the DNA (Gardes & Bruns 1993). Samples were then dried on a forced-air dryer at 45 °C for approximately 24 h and then stored in plastic bags with silica gel. Specimens are accessioned at the following fungal herbaria: the Florida Museum of Natural History (FLAS) at the University of Florida and the Farlow Herbarium at Harvard University (FH) in the USA, the Herbario del Museo Botánico de Córdoba (CORD) in Argentina and the Museo Nacional de Historia Natural de Chile (SGO) in Chile. Additional specimens were borrowed from the J.F. Bell Museum of Natural History (MIN) and the Ada Hayden Herbarium (ISC).

Morphological analysis

Dried material was rehydrated in DI water, hand-sectioned with a razor blade and mounted in water, 3 % KOH, cotton blue in lactic acid, or Melzer's reagent. Images were captured using a Q-Imaging Micropublisher v. 3.3 RTV digital camera (British Columbia, Canada) mounted on a Nikon Optiphot light microscope. Images were edited in Adobe Illustrator v. CS5.1 (San Jose, California) to increase contrast and remove background objects. Relevant morphological characters, including excipular tissues, ascospores, spore ornamentation, asci and paraphyses, were studied and their sizes assessed based on 20 individual measurements at various magnifications. Measurements include the range and average values for most features and the length-to-width ratio (Q) for spores. For scanning electron microscopy, a piece of apothecium was rehydrated in 2.5 % KOH, dehydrated in an ethanol series to 100 %, mounted on carbon tape on aluminium stubs, sputter coated with palladium and viewed with a Hitachi S3500N Variable Pressure Scanning Electron Microscope at 10 KV at the University of Minnesota Imaging Center. Images were digitally captured. Microscopic features were compared with the known species of *Gymnohydnotrya* and *Underwoodia* based on original descriptions and type specimens when available.

Molecular and phylogenetic analyses

Clean fungal tissues were taken from 32 fresh or dried specimens. DNA was then extracted using a modified CTAB method (Gardes & Bruns 1993). Polymerase chain reactions (PCR) of the nuclear rDNA ITS1-5.8S-ITS2 region (ITS) were performed using forward primer ITS1F (Gardes & Bruns 1993) and reverse primer ITS4 (White et al. 1990). PCR of the large rRNA subunit (28S) was performed with forward primer LROR (Hopple Jr. & Vilgalys 1994) and reverse primer LR5F (Tedersoo et al. 2008). PCR of the second largest subunit of RNA polymerase II (*rpb2*) was amplified using the forward primer P6Fa and reverse primer 7Ra (Hansen et al. 2005). PCR of the translation elongation factor 1- α (*EF1 α*) was performed using the forward primer Df and reverse primer 2212r (Matheny et al. 2007, Rehner & Buckley 2005). All genes were amplified with Phusion Hot Start Flex DNA Polymerase kit using the manufacturer's protocol (New England BioLabs Inc., Ipswich, Massachusetts).

PCR products were visualized on 1.5 % agarose gels stained with SYBR Green I (Molecular Probes, Eugene, Oregon). Amplicons were cleaned with EXO (Exonuclease I) and SAP (shrimp alkaline phosphatase) enzymes (Werle et al. 1994) and

Table 1 Taxa used in this study with corresponding herbarium and GenBank accession numbers.

Taxon	Synonym	Geographi- cal origin	Collector number	Herbarium (accession number)	GenBank Accession Number				Reference
					ITS	28S	EF1	RPB2	
<i>Balsamia nigrans</i>	–	USA	MES-3108	FLAS-F-60811	–	–	MK873424	MK825506	This publication
<i>Balsamia quericicola</i>	–	USA	MES-84	FLAS-F-58857	–	–	GU596458	JO954467	Bonito et al. (2013)
<i>Choromyces alveolatus</i>	–	USA	MES-97	DUKE-0348839	–	–	–	JO954470	Bonito et al. (2013)
<i>Discina</i> sp.	–	USA	04-mwb-050917 (MB)	FLAS-F-61956	–	–	–	–	This publication
<i>Discofotis venosa</i>	–	Unknown	AFTOL-ID 179	OSC-100045	–	–	DQ471060	DQ470892	Hansen et al. (2013)
<i>Geomorium australianum</i>	–	Australia	–	OSC-130601	–	–	–	–	Bonito et al. (2013)
<i>Geomorium beatonii</i>	<i>Gymnohydrotia australiana</i>	Australia	JT-19760	OSC	–	–	–	–	Bonito et al. (2013)
<i>Geomorium echinulatum</i>	<i>Gymnohydrotia australiana</i>	Australia	JT-28380	OSC	–	–	–	–	Bonito et al. (2013)
<i>Geomorium fuegianum</i>	<i>Underwoodia beatonii</i>	Australia	AWC-4529	OSC-80059	–	–	–	–	Bonito et al. (2013)
	<i>Gymnohydrotia echinulata</i>	Chile	MES-2420	SGO, FLAS-F-63308, FH	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-2510	SGO, FLAS-F-63310, FH	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-2554	SGO, FLAS-F-63311	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-1502	SGO, FLAS-F-63304	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-1663	SGO, FLAS-F-62857	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-1560	SGO, FLAS-F-62856	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-1547	SGO, FLAS-F-62860	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-1520	SGO, FLAS-F-63305	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	MES-1521	SGO, FLAS-F-62855	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	CT-4392	SGO, FLAS-F-62904	–	–	–	–	This publication
	<i>Underwoodia fuegiana</i>	Chile	CT-4268	CORD-C00006471, FLAS-F-62903	–	–	–	–	This publication
<i>Geomorium furciae</i> (TYPE)	–	Argentina	DHP-CH-126	SGO (holotype), FLAS-F-62848, FH-00290548 (isotypes)	KY962388	MK430985	MK873420	–	This publication
<i>Geomorium gamundiae</i>	–	Argentina	AM-AR17-28	CORD-C00006470, FLAS-F-62850	–	–	–	–	This publication
<i>Geomorium gamundiae</i> (TYPE)	–	Argentina	MES-577	CORD-C00006469 (holotype), FLAS-F-62851, FH (isotypes)	KY462684	–	–	MK825485 MK825486	This publication
<i>Geomorium geodon</i>	<i>Underwoodia singeri</i>	Argentina	MES-1239	CORD-00006473, FLAS-F-62854	KY462444	MK430977	MK873411	MK825489	This publication
	<i>Underwoodia singeri</i>	Chile	MES-2377	SGO, FLAS-F-62853	MK430951	MK430980	MK873414	–	This publication
<i>Geomorium geodon</i> (TYPE)	<i>Underwoodia singeri</i>	Chile	MES-2362	SGO (holotype), FLAS-F-62852 (isotype)	MK430942	MK430979	MK873413	MK825492	This publication
<i>Geomorium singeri</i>	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-2266	SGO	MK430931	MK430974	–	MK825488	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-2917	SGO, FLAS-F-63075	MK430949	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-1073	SGO, FLAS-F-63313, FH	MK430934	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-2440	FLAS-F-63075	MK430944	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-161	FH-00940351	–	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-2390	SGO, FLAS-F-63307	MK430943	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-2572	SGO, FLAS-F-62861	MK430947	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	CT-4611	SGO, FLAS-F-62902	MK430930	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Chile	MES-2297	SGO	MK430941	–	–	–	This publication
	<i>Underwoodia singeri</i> var. <i>singeri</i>	Argentina	MES-1986	CORD-C00006472, FLAS-F-62858	–	–	–	–	This publication
<i>Gyromitra ancilis</i>	–	USA	MES-3058 (MB)	FLAS-F-61938	–	–	–	–	This publication
<i>Gyromitra californica</i>	–	Unknown	AFTOL-ID 176	OSC-100068	–	–	–	–	This publication
<i>Gyromitra sphaerospora</i>	–	USA	BD-521	MIN-925941	–	–	–	–	Hansen et al. (2013)
<i>Helvella dryophila</i>	–	USA	MES-218	FLAS-F-63279, FH-01146490	–	–	–	–	This publication
<i>Helvella elastica</i>	–	USA	RH-1696	FLAS-F-63280	–	–	–	–	This publication
<i>Helvella</i> sp.	–	USA	MES-3131	FLAS-F-60833	–	–	–	–	This publication
	–	USA	1599/RH-1703	FLAS-F-63281	–	–	–	–	Bonito et al. (2013)
<i>Hydnortia cubispora</i>	–	Canada	DHP-05-605	FH-00290174	–	–	–	–	This publication
<i>Labyrinthomyces</i> sp.	–	Australia	JT-27750	OSC	–	–	–	–	This publication
<i>Morchella americana</i>	–	USA	91-51	ISC-425665	–	–	–	–	This publication
<i>Morchella angusticeps</i>	–	USA	89-37	ISC-426682	–	–	–	–	Hansen et al. (2013)
<i>Morchella echespera</i>	–	USA	MES-3066 (MB)	FLAS-F-61946	–	–	–	–	Bonito et al. (2013)
<i>Morchella punctipes</i>	–	USA	91-40	ISC-422395	–	–	–	–	This publication
	–	USA	GS-13	ISC-453748	–	–	–	–	This publication
<i>Morchella vulgaris</i>	–	USA	MES-3065 (MB)	FLAS-F-61945	–	–	–	–	This publication
<i>Nothofagus cf. thaxteri</i>	–	Argentina	N-10 (EN-60)	CORD-C00006474	–	–	–	–	Bonito et al. (2013)
<i>Tuber magnatum</i>	–	Italy	GB-12	unknown	–	–	–	–	This publication
<i>Underwoodia columnaris</i>	–	USA	MES-724	FLAS-F-58861, ISC-F-0100301	–	–	–	–	Bonito et al. (2013)
<i>Verpa bohemica</i>	–	USA	93-2	ISC-434936	–	–	–	–	This publication

sequenced by Genewiz (South Plainfield, New Jersey) or Eurofins Genomics (Louisville, Kentucky). Sequences were then edited with Sequencher v. 5.0.1 (Gene Codes Inc., Ann Arbor, Michigan). Additional sequences from Hansen & Pfister (2006) and Bonito et al. (2013) were downloaded from the GenBank NCBI database (Clark et al. 2016). An alignment for each locus was created in Mesquite v. 3.2 (Maddison & Maddison 2018) with the aid of Muscle v. 3.8.31 (Edgar 2004). Ambiguous regions in the multi-locus alignment were removed with Gblocks 0.91b (Castresana 2000) using the default parameters and 'with-half-gap' option, which removes columns where characters are missing in more than half of all the taxa. Because no incongruence was detected among partitioned 28S, *rpb2*, and *EF1 α* genes, we concatenated them with Super-Aligner code (Mujic

et al. 2019) into a single matrix. The concatenated alignment is deposited in TreeBASE under submission 24370. However, the ITS region was too divergent to align across the various families of *Pezizales* so this locus was examined independently. The ITS alignment was edited manually to exclude ambiguous regions. The ITS alignment was deposited in TreeBASE under submission 24783.

The concatenated multi-locus alignment (28S, *rpb2*, and *EF1 α*) was analysed with Maximum Likelihood (ML) and Bayesian methods. Both were performed using the Cyberinfrastructure for Phylogenetic Research Science Gateway (Cipres) 3.1 (Miller et al. 2010). Maximum Likelihood was run using RAxML v. 8.2.10 (Stamatakis 2014) with 1000 bootstrap iterations and the GTR-GAMMA model. Partitioned ML analysis was also run with the

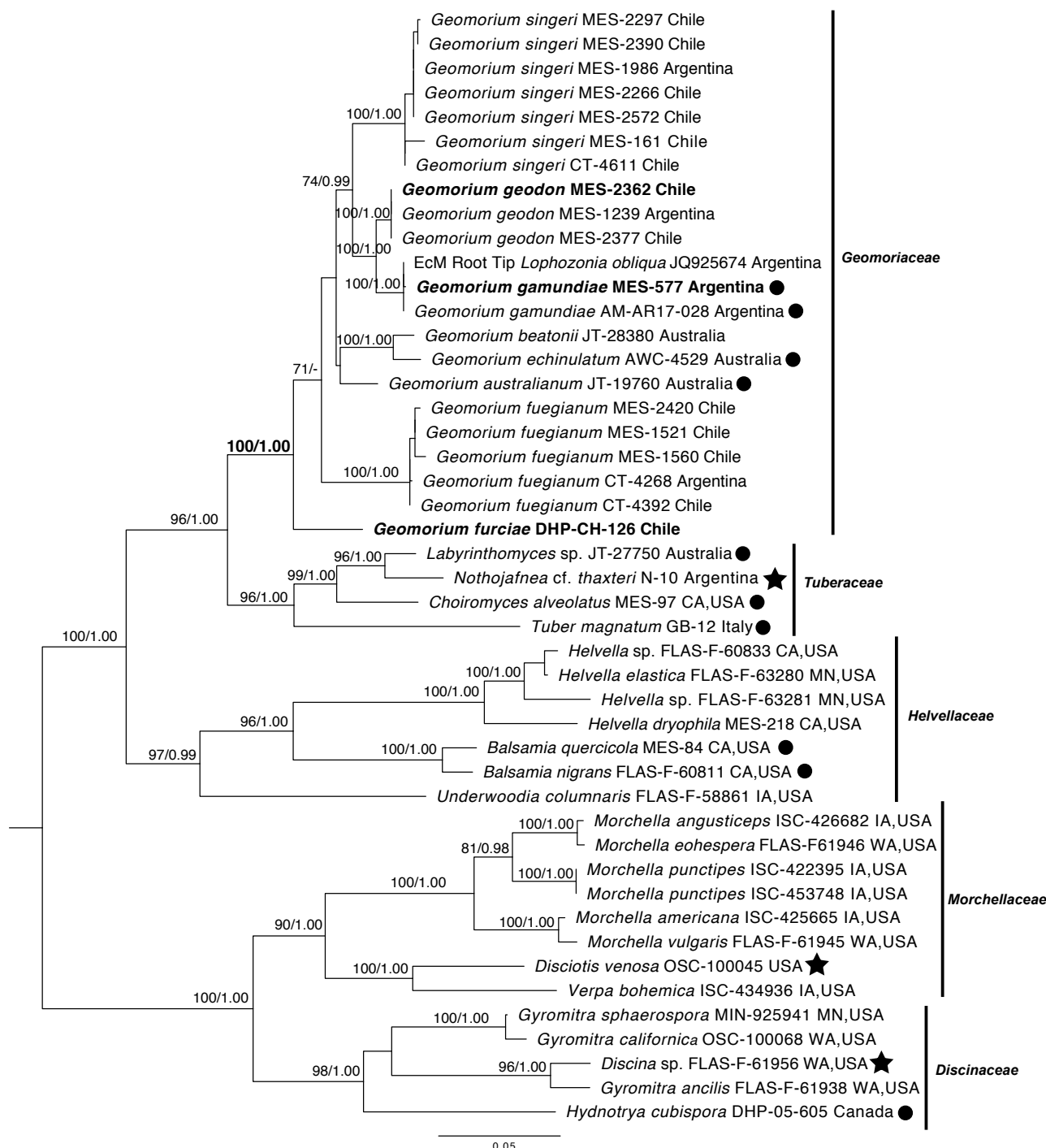


Fig. 1 Phylogram of *Geomoriaceae* and related species obtained from Maximum Likelihood analysis of three concatenated loci (28S rDNA, *rpb2*, *EF1 α*). Numbers above branches represent ML bootstrap values followed by BP probabilities. ML bootstrap values $\geq 70\%$ and BPP ≥ 0.95 are shown here. Sequences of type specimens are highlighted in **bold**. Specimen voucher numbers and locations are indicated after species names. Symbols following taxa symbolize ascoma form (● = hypogaeous and truffle-like; ★ = epigeous and cupulate; no symbol = epigeous and columnar apothecium). Bar represents the expected nucleic acid changes per site.

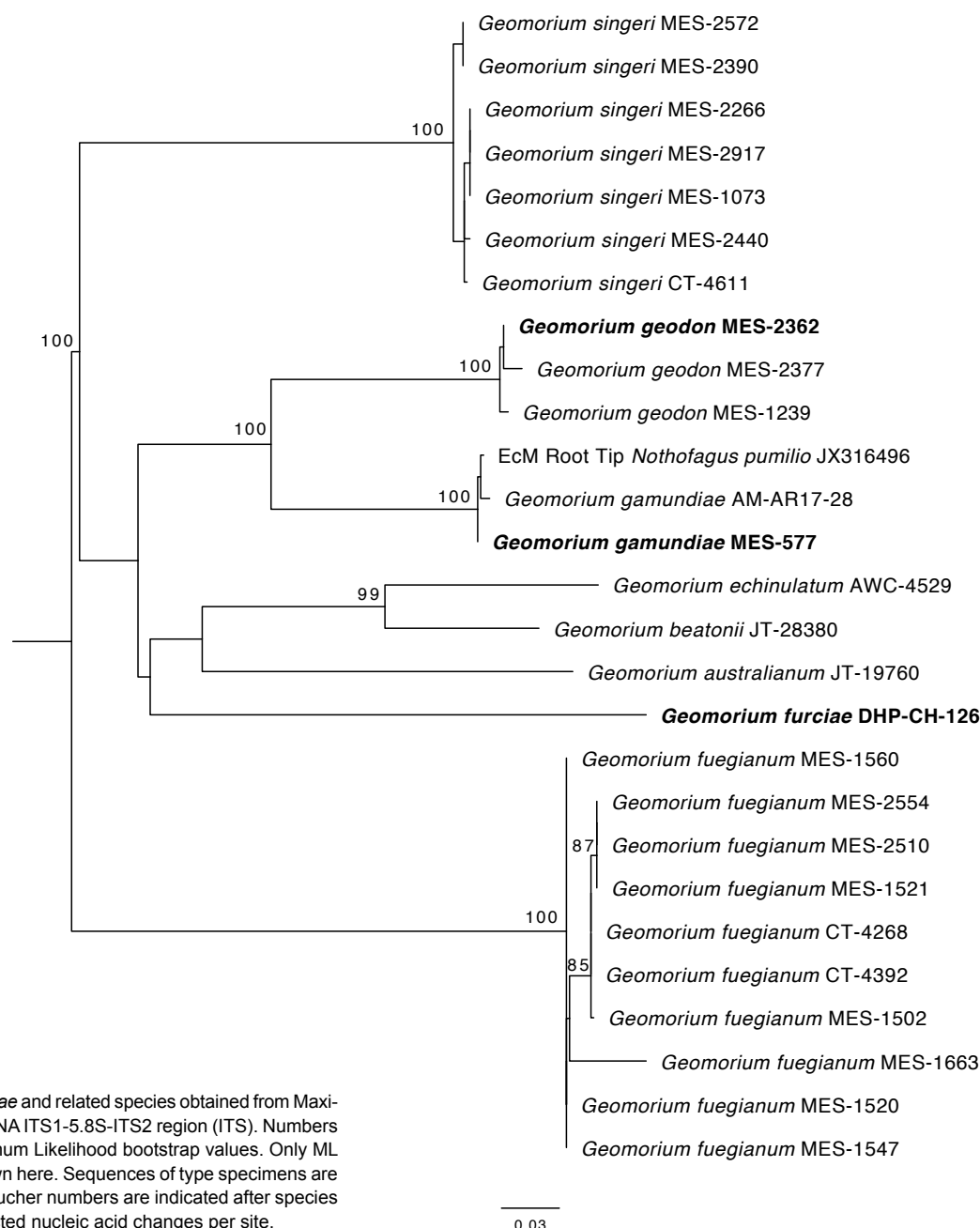


Fig. 2 Phylogram of *Geomoriaceae* and related species obtained from Maximum Likelihood of the nuclear rDNA ITS1-5.8S-ITS2 region (ITS). Numbers above branches represent Maximum Likelihood bootstrap values. Only ML bootstrap values $\geq 70\%$ are shown here. Sequences of type specimens are highlighted in **bold**. Specimen voucher numbers are indicated after species names. Bar represents the expected nucleic acid changes per site.

same model and parameters. The ITS alignment was analysed separately with the same Maximum Likelihood parameters. The concatenated alignment was then partitioned into 28S, *rpb2*, and *EF1 α* matrices for Bayesian analysis. Evolutionary models for each partition were estimated independently by jModelTest2 v. 2.1.6 (Darriba et al. 2015). The GTR + I + G model was selected for all partitions. Bayesian inference was calculated using MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001) with a chain length of 10 M generations and a sampling frequency of 1000 with the first 25 % of samples discarded as the burn-in. The rest of the parameters were set to default. Resulting phylogenetic trees for both ML and Bayesian analyses were visualized and rooted in FigTree v. 1.4.3 (Rambaut 2009). The multi-locus phylogeny was rooted with species from the *Morchellaceae* and *Discinaceae* as the outgroup, whereas the ITS tree was mid-point rooted. Nodes were considered strongly supported when the ML bootstrap values were $\geq 70\%$ and the Bayesian posterior probabilities were ≥ 0.95 . Final trees were edited in Adobe Illustrator v. CS5.1 (San Jose, California).

Divergence time estimation

Molecular divergence time analyses were performed in Bayesian Evolutionary Analysis by Sampling Trees (BEAST) v. 1.8.3 (Drummond et al. 2012) using the same concatenated alignment as above (28S, *rpb2*, and *EF1 α*). Temporal calibration was calculated following Bonito et al. (2013) by fixing the absolute rate of molecular evolution for 28S at 6.561024 substitutions per site per MY (Otálora et al. 2010) and estimating the evolutionary rates of *rpb2* and *EF1 α* relative to the fixed 28S rate with a relaxed clock model and an uncorrelated exponential prior distribution. For comparison purposes we also performed another dating analysis using a log-normal distributed clock model and two secondary calibration points based on the phylogenetic analysis of *Morchellaceae* by O'Donnell et al. (2011). These were calculated from primary fossil data:

1. the divergence time of the genus *Morchella* from its epigeous sister genera *Verpa* and *Disciotis* (normal distribution with mean = 129.6 MY and SD = 3);
2. the most recent common ancestor of subg. *Elata* (normal distribution with mean = 73.5 MY and SD = 3).

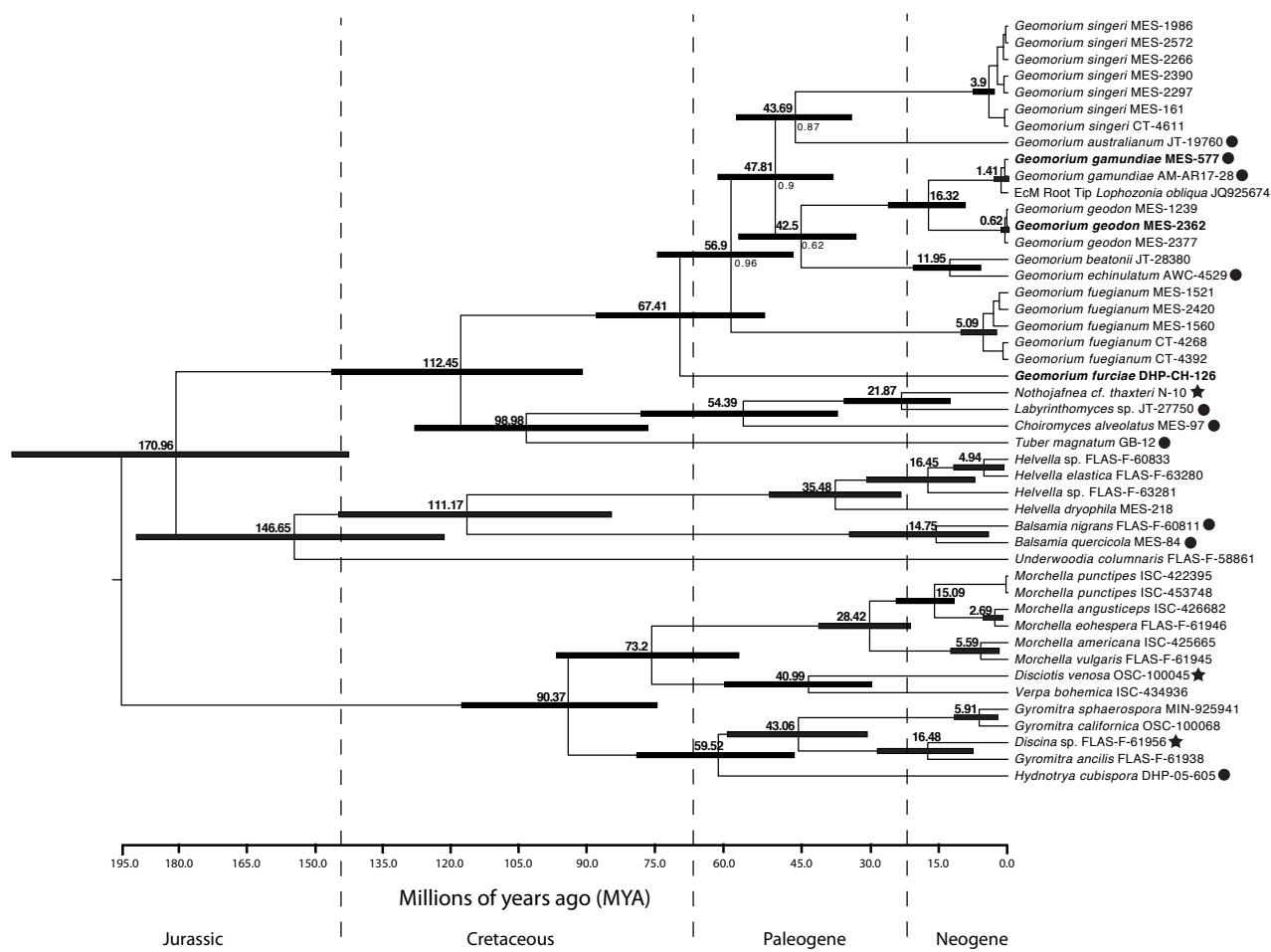


Fig. 3 Chronogram estimation of *Geomoriaceae* and related species obtained from BEAST analysis of three concatenated genes (28S rDNA, *rpb2*, *EF1α*). Number above the node represents the mean estimated divergence time with a black bar representing the 95 % confidence interval. All nodes receive maximum Bayesian Posterior Probability unless noted below the node. Sequences of type specimens are highlighted in **bold**. Specimen voucher numbers are indicated after species names. Symbols following taxa symbolize ascoma form (● = hypogeous and truffle-like; ★ = epigeous and cupulate; no symbol = epigeous and columnar apothecium).

In both analyses, the sequence data were partitioned by gene region using an unlinked clock and the GTR + G + I substitution model. The coalescent (constant rate) model was employed to account for the intragenetic variation of species with linked trees and a random sampling tree for each run. Two MCMC runs were performed in parallel with a chain length of 100 M generations, sampling every 10 000th state and with the first 25 % of samples discarded as burn-ins. The resulting trees from both runs were combined to generate a maximum clade credibility tree in TreeAnnotator v. 1.8.3. Run convergence, stationarity, and effective sample size were verified in Tracer v. 1.6 (Rambaut et al. 2014).

RESULTS

The concatenated multi-locus alignment was comprised of 46 specimens and a total of 108 sequences (1844 nucleic acid sites, 28S: 592 sites; *rpb2*: 629 sites; *EF1α*: 623 sites). The ITS alignment was comprised of sequences from 28 specimens with 539 nucleic acid sites. Sample information and GenBank accession numbers are listed in Table 1. The most likely tree from the multi-locus phylogenetic analyses (Fig. 1) provides significant new information about the genus *Underwoodia* and the *Gymnohydnotrya* lineage.

First, our multi-locus phylogeny (Fig. 1) provides strong support for a distinct lineage of Southern Hemisphere fungi. *Underwoodia columnaris*, the type of the monotypic genus *Underwoodia*, is a

lineage distinct and distant from the Southern Hemisphere fungi where the name '*Underwoodia*' has been applied to several species, including *U. singeri* and *U. fuegianum*. *Underwoodia columnaris* is strongly supported within the *Helvellaceae*, as a sister to *Helvella* and *Balsamia*. In our analyses, *Gymnohydnotrya* is nested with *U. singeri* and *U. fuegianum* within the Southern Hemisphere lineage that also includes Spegazzini's *Geomorium fuegianum*, type of the genus *Geomorium*. The name *Geomorium* dates back to Spegazzini (1922) and therefore is the oldest legitimate name for this group. In order to recognize this group as monophyletic, we transfer all described species in the */gymnohydnotrya* lineage to the genus *Geomorium*. Since members of this group are morphologically and phylogenetically distinct from their relatives in the *Tuberaceae* and the *Helvellaceae*, we also erect a new family *Geomoriaceae*. Taxonomic treatment and a key to the species of *Geomoriaceae* are discussed and provided below.

Second, multiple specimens identified as *Underwoodia singeri* formed two distinct non-sister lineages in both multi-locus and ITS phylogenies (Fig. 1, 2). We believe that the two lineages likely correspond to the two described varieties of *U. singeri* (*U. singeri* var. *singeri* and *U. singeri* var. *fulvostipitata*), but we were not able to locate the type of the variety *U. singeri* var. *fulvostipitata* to confirm this hypothesis. Based on our morphological analysis of the type specimen of *U. singeri*, we consider this typical and more common variety as *Geomorium singeri*. Lacking material of *U. singeri* var. *fulvostipitata*, we name the

second and more rare species as *Geomorium geodon* in order to avoid future nomenclatural confusion about the potential identity of *U. singeri* var. *fulvostipitata*.

Third, two additional unnamed South American species, one partially epigeous and one fully hypogeous, were resolved within the /gymnohydnotrya lineage. The hypogeous species is distantly related to *Gymnohydnotrya* s.str., as represented by two of the three described taxa (*G. echinulata* and *G. australiana*). We describe this hypogeous taxon below as *Geomorium gamundiae* sp. nov. The partially epigeous taxon is sister to the rest of the /gymnohydnotrya lineage, albeit with low bootstrap support. This new taxon is resolved with strong support within the *Geomorium* clade and is therefore described below as *Geomorium furciae*.

Lastly, our dating analysis (Fig. 3) suggests that *Helvellaceae* diverged from the /Geomoriaceae-Tuberaceae superclade in the mid-Jurassic, c. 171 MYA. The *Geomoriaceae* clade arose sometime between 112 and 67 MYA. *Geomorium furciae* was putatively the first to diverge from other species in the family (in the late-Cretaceous, c. 67 MYA). All other species in the genus *Geomorium*, i.e., *G. australianum*, *G. beatonii*, *G. echinulatum*, *G. fuegianum*, *G. gamundiae*, *G. geodon*, and *G. singeri*, evolved more recently in either the Paleogene or Neogene period, c. 75–12 MYA. A supplementary comparison analysis based on the secondary fossil calibration of O'Donnell et al. (2011) suggests that the *Geomoriaceae* clade arose much earlier (c. 227–124 MYA) and that *Helvellaceae* diverged from the /Geomoriaceae-Tuberaceae superclade at c. 353 MYA (Fig. S1).

Although the phylogenies from the Maximum Likelihood analysis and the molecular dating analysis (BEAST) resolve a similar topology with similar support values (Fig. 1–3), there is a minor incongruency regarding the placement of *Geomorium australianum*. The multi-locus and ITS ML analyses place *G. australianum* in the same clade as the other Australasian taxa, *G. beatonii* and *G. echinulatum*, forming an Australasian clade (Fig. 1, 2). The BEAST analysis, however, places *G. australianum* as sister to *G. singeri* (Fig. 3). It is important to note that both placements of *G. australianum* receive low support values (38 % in multi-locus ML, 65 % in ITS ML, and 0.86 in BEAST).

TAXONOMY

Geomoriaceae Kraisit., Pfister & M.E. Sm., *fam. nov.* — MycoBank MB828305

Etymology. Based on the type genus *Geomorium* described by Spegazzini (1922).

Ascomata either a modified columnar apothecium or an exothecium, 1–40 mm broad. Young ascomata typically white or pale tan but often changing to brown, purple, or black as the hymenium matures. Outer excipulum and stipe present only in the epigeous taxa, lacking hairs, outer excipulum palisade-like, made up of isodiametric cells, perpendicular to the outer surface. *Paraphyses* slightly or greatly exceeding the asci in length. *Asci* cylindrical, lacking opercula, spores uniseriate to biseriate. *Ascospores* hyaline or yellowish, ellipsoid to subglobose, and ornamented with warts ranging from small in the epigeous taxa, to large in the hypogeous taxa. Ectomycorrhizal, found in forests or at forest edges, known only from the Southern Hemisphere.

Type genus. *Geomorium* Speg., Anales Soc. Ci. Argent. 94(1–2): 79. 1922.

Geomorium Speg. emend. Kraisit., Pfister & M.E. Sm.

Ascomata either a modified columnar apothecium or an exothecium, 1–40 mm broad. Young ascomata typically white or pale tan but changing to brown, purple, or black as the hymenium

matures. In the epigeous species, lacking hairs, outer excipulum a palisade-like layer, 50–350 µm thick, made up with isodiametric cells, 7–20 µm diam, perpendicular to the outer surface. *Paraphyses* typically slightly exceeding the length of asci, although paraphyses far exceed the asci in some hypogeous taxa. At maturity in some taxa paraphyses have dark granular inclusions in the apical cells. *Asci* cylindrical, spores uniseriate in epigeous taxa, but often irregularly uniseriate or biseriate in hypogeous taxa, lacking opercula. *Ascospores* typically hyaline but occasionally yellowish, ellipsoid to subglobose, and ornamented with warts that range from small in the epigeous taxa to large in the hypogeous taxa. Ectomycorrhizal, found in forests or at forest edges, fruiting on the ground in association with *Nothofagaceae* (South America and Australasia) or *Myrtaceae* (Australasia) and perhaps with other host plants. Known only from the Southern Hemisphere.

Type species. *Geomorium fuegianum* Speg., Anales Soc. Ci. Argent. 94 (1–2): 79. 1922.

Geomorium fuegianum Speg., Anales Soc. Ci. Argent. 94 (1–2): 79. 1922 — Fig. 4c, d

Synonyms. *Helvella fuegiana* (Speg.) Eckblad, Nytt Mag. Bot. 15(1–2): 92. 1968.

Underwoodia fuegiana (Speg.) Gamundi, Darwiniana 11(3): 419. 1957.
? *Underwoodia fuegiana* var. *cabrinii* Raithelh., Metrodiana Sonderh. 2: 20. 1983.

Typus. ARGENTINA, South east Tierra del Fuego, on soil among logs close to Río Grande (Ad humum inter truncus dejectos secus Río Grande, Fuegia austro-orientali), Mar. 1921, C.L. Spegazzini (not examined).

Ascomata 50–250 × 10–30 mm, a modified columnar apothecium, fleshy, ranging in shape from cylindrical to clavate, broadly cavitated and ridged on the outside or longitudinally wrinkled, mature hymenium olivaceous brown to black, covering the upper half, below white and sterile. Outer excipulum lacking hairs, palisade-like, 145–174 µm thick, composed of cells 5.8–11.6 µm wide, perpendicular to the outer surface. *Paraphyses* filiform, irregularly septate, exceeding the asci by 30–50 µm, the apex rounded and 4–6 µm wide, upper cells filled with brown-olivaceous granules at maturity. *Asci* 350–400 × 14–16 µm, usually with eight spores, tapering at base, cylindrical, dextrinoid when young but no reactions to Melzer's when mature. *Ascospores* 24–26 × 11–14 µm, av. 25 × 12.5 µm, Q = 2, uniseriate, ellipsoid, hyaline, always with a central guttule, smooth when young but later covered with irregular warts, 3–4 × 1–3 µm.

Habit, Habitat & Distribution — Solitary or occasionally in clusters, fruiting directly on soil among leaf litter. Found in *Nothofagaceae* forests in both Chile and Argentina.

Specimens examined. ARGENTINA, Tierra Del Fuego, Jeujepen, disturbed forest dominated by *Nothofagus pumilio* (54 33 37.8S – 67 12 49.68W), 150 m above sea level, on soil, 2 Apr. 2015, C. Truong CT-4268 (FLAS-F-62903, CORD-C00006471); Lapataia, N.E. Lago Roca, soil in a meadow, 14 Mar. 1975, Gamundi, Giaioti, Horak (Gamundi 49), det. Irma Gamundi (FH- 00290550). — CHILE, Tierra del Fuego, Parque Karukinka, Mar. 2012, Giuliana Furci (FH-00995013); Magallanes, Magallanes Forest Reserve, on Sendero de Chile across the street from the parking lot above the park ranger station (53 8 38.9S – 71 0 12W), 349 m above sea level, in a *Nothofagus pumilio* forest with *N. betuloides* at forest edges, on soil, 4 Apr. 2017, M.E. Smith MES-2420 (SGO); Magallanes Forest Reserve near the park ranger station (53 8 34.6S – 71 0 17.5W), 343 m above sea level, in a *Nothofagus pumilio* forest with *N. betuloides* at forest edges, on soil, 6 Apr. 2017, M.E. Smith MES-2510 (SGO); same location, in a *Nothofagus pumilio* forest, 7 Apr. 2017, M.E. Smith MES-2554 (SGO); Osorno, Puyehue National Park, below Antillanca on the edge of the road, near a big flat wash area, 991 m above sea level, near *Nothofagus pumilio*, on soil, 3 May 2016, R. Swenie MES-1502 (SGO); *ibid.*, M.E. Smith MES-1509 (SGO); *ibid.*, P. Sandoval MES-1520 (SGO); *ibid.*, MES-1521 (FLAS-F-62855, SGO-169909); *ibid.*, G. Furci MES-1547 (SGO); *ibid.*, A.B. Mujic MES-1560 (FLAS-F-62856, SGO-169907); Puyehue National Park, near bottom of Sendero Mirador el



Fig. 4 Morphological comparison of: a–b. *Geomorium singeri*; c–d. *Geomorium fuegianum*. a. Aggregated *G. singeri* ascomata in the field as they emerge from the soil (CT-4611); b. hymenium of *G. singeri* (MES-2297) showing mature ascospores inside an ascus exceeded by paraphyses that are clear to pale yellow and wrinkled at the apices; c. fresh dissection of *G. fuegianum* ascomata (MES-2420) showing hollow and convoluted chambers on the inside and black hymenium on the outside; d. hymenium of *G. fuegianum* (MES-1560) showing mature ascospores inside an ascus and paraphyses with dark brown pigments at the apices. — Scale bars: a = 2 cm; b, d = 20 μ m; c = 3 cm.

Puma, near *Nothofagus dombeyi*, on soil, 5 May 2016, R. Swenie MES-1663 (FLAS-F-62857, SGO-169906); Aysén, Villa Ortega, camino al Cerro Rosado (45 22 54.012S – 72 5 27.996W), near *Nothofagus pumilio*, on soil, 29 Apr. 2016, C. Truong CT-4392 (FLAS-F-62904, SGO-169910).

Notes — Our description of *G. fuegianum* is taken from the original description (Spegazzini 1922), a study by Gamundi (1957), and review of our fresh specimens. Morphological characters of our specimens match well with the original description of *G. fuegianum*, particularly the hollow internal structure and the ridged and wrinkled ascomata that are always white at the base but darker at the apex. This species is widespread in Patagonia and all of our collections have very similar ITS sequences. Since our phylogeny shows that this species is not closely related to *U. columnaris*, we recognize it as *G. fuegianum* under the name bestowed by Spegazzini (1922). We find no evidence that *U. fuegiana* var. *cabrini* is a unique taxon, except for having asci 5–10 μ m wider than recorded here (Raithelhuber 1983). The description is brief and lacking an authentic specimen; there is no way to verify its identity. Thus, we consider it here as a synonym of *G. fuegianum*.

Geomorium australianum (B.C. Zhang & Minter) Kraisit., Pfister & M.E. Sm., *comb. nov.* — MycoBank MB828309

Basionym. *Gymnohydnotrya australiana* B.C. Zhang & Minter, Mycol. Res. 92(2): 193. 1989.

Typus. AUSTRALIA, Victoria, Rye, found under *Pittosporum* spp. (Asteraceae) and *Leptospermum laevigatum* (Myrtaceae), Jan. 1984, anonymous, holotype K spirit collection no. 1032 (not examined).

Specimens examined. AUSTRALIA, Victoria, East Gippsland, Cabbage Tree Forest Management block, Towser Link Track, 0.7 km NE junction Dorthat Rd., Claridge site R16, 19 Apr. 1996, S. Bobbin AWC-408 (OSC-130601); East Gippsland, rich forest management block, Jack Rd., Claridge site R19, 25 July 1996, A.W. Claridge JT-19760 (OSC).

Notes — The type specimen is preserved in liquid and was not available for molecular or morphological studies (Zhang & Minter 1989). Nevertheless, morphological characters of the specimens we examined (cited above) match well with the original description by Zhang & Minter (1989). Sequences from specimen JT-19760 (Bonito et al. 2013) were included in our phylogenetic analyses and the results indicate that *Gymnohydnotrya australiana* is nested inside the genus *Geomorium* (Fig. 1, 3). Thus, we transfer this species to the genus *Geomorium*.

Geomorium beatonii (Rifai) Kraisit., Pfister & M.E. Sm., *comb. nov.* — MycoBank MB828308

Basionym. *Underwoodia beatonii* Rifai, Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., sect. 2, 57(3): 69. 1968.

Synonym. *Helvella beatonii* (Rifai) Harmaja, Karstenia 14: 103. 1974.

Typus. AUSTRALIA, Victoria, close to Anglesea, on land, 18 July 1964, G. Beaton 215, isotype, MELU-102839 (examined).

Ascomata 70 × 10 mm, gregarious to clustered, erect, tapering upwards, slightly curved and horn-like, internally hollow and lacunose, filled with large longitudinal alveole-like cavities. Stipe occupying the lower half of the ascoma, fluted, minutely downy, creamy white. Outer excipulum lacking hairs, palisade-like layer up to 80 µm thick, composed of septate cells 8–20 µm wide, perpendicular to the outer surface. Hymenium covering the upper half of the ascoma, smooth, greyish brown, becoming blackish when dried, about 360 µm thick. *Paraphyses* slender, clavate to subclavate, often anastomosing near the base, sparingly septate, distinctly enlarged and pigmented with yellow at the apex, 5–7.2 µm diam. *Asci* 320–400 × 16–20 µm, cylindrical, slightly tapering downwards, 8-spored, thick-walled. *Ascospores* 22.7–25.4 × 10.9–12.5 µm excluding ornamentations, uniseriate, ellipsoidal, hyaline to subhyaline, usually containing one large central guttule and two smaller ones, at maturity covered by rounded to irregularly-shaped warts less than 1.8 µm diam.

Habit, Habitat & Distribution — On soil, known only from Australia.

Additional specimen examined. AUSTRALIA, Victoria, Deep Lead Education Reserve, unnamed road 800 m NE of Western Highway 653320 E, 5902938 N, Claridge Site 216, EVC SW2, 240 m above sea level, in flat, edge-rich woodland, near *Acacia* sp., *Eucalyptus leucoxylon*, and *E. microcarpa*, on soil, 29 Aug. 2014, J. Trappe JT-28375 (OSC).

Notes — Although this species is morphologically similar to *Geomorium fuegianum* and *G. singeri*, all of our molecular analyses suggest that the closest relative to *G. beatonii* is *G. echinulatum*, a hypogeous truffle-like fungus. Nevertheless, all four species are nested in the *Geomoriaceae* clade (Fig. 1–3). It is therefore appropriate to transfer this species to the genus *Geomorium*. Our description is based on the original description by Rifai (1968) and our examination of the type specimen (MELU-102839). Morphology of the type specimen also matches perfectly with our collection of *G. beatonii* (JT-28375). According to records at the Atlas of Living Australia (<https://biocache.ala.org.au>), *G. beatonii* is rare and has been reported only occasionally from South Australia, Victoria, and Tasmania.

Geomorium echinulatum (G.W. Beaton) Kraisit., Pfister & M.E. Sm., *comb. nov.* — MycoBank MB828330

Basionym. *Sphaerozone echinulatum* G.W. Beaton, Trans. Brit. Mycol. Soc. 71(1): 165. 1978.

Synonym. *Gymnohydnotrya echinulata* (G.W. Beaton) B.C. Zhang & Minter, Mycol. Res. 92(2): 196. 1989.

Typus. AUSTRALIA, Victoria, Mait's Rest near Apollo Bay, 12 July 1976, holotype, *G. Beaton* 314a. MELU-103219 (examined).

Additional specimen examined. AUSTRALIA, New South Wales, Nungatta State Forest, unnamed track, 1.9 km NE of Junction Nungatta Road and Poole Road, 0.1 km SE Junction Poole Road, on sheltered slope with *Eucalyptus cypellocarpa* and *E. muelleriana* on the plot, Claridge site 21, 1 June 2001, W. Colgan III AWC-4529 (OSC-80059) (immature).

Notes — In addition to our molecular results based on the specimen listed above, we hypothesize that *Gymnohydnotrya echinulata* (= *Sphaerozone echinulatum*) belongs to the genus *Geomorium* because of the morphological similarity between this species and *Geomorium beatonii*. According to Rifai (1968), *G. beatonii* has large 'nematode-like cells' embedded in the flesh. The original description of *Sphaerozone echinulatum* indicates that it has a similar cell type (Beaton & Weste 1978), which is an unusual cell type for fungi in the *Pezizales*. The type specimen was borrowed from MELU and examined. The general morphology matches perfectly with the detailed description provided by Beaton & Weste (1978, 1982) and Zhang & Minter (1989). Molecular analysis of the immature specimen

cited above and putatively identified as *Gymnohydnotrya echinulata*, places it close to *Geomorium beatonii* in the phylogenies (Fig. 1, 3). This supports our hypothesis that *G. beatonii* and *G. echinulatum* are close relatives. Furthermore, collections of these two species were only found in southern Australia, suggesting that they are likely endemic to this region. Additional molecular data from fresh mature collections are needed to verify the relationships of this taxon.

Geomorium furciae Kraisit., Pfister & M.E. Sm., *sp. nov.* — MycoBank MB828332; Fig. 5

Etymology. Named in honour of Giuliana Furci in recognition of her pioneering work on fungal conservation in Chile and her love of Patagonian fungi.

Typus. CHILE, Aysén, near the mouth of the Río Melimoyu Sur, along the Tres Lagunas trail in old growth *Nothofagus dombeyi* forest at the top of the hill near Laguna Mallín, 11 Mar. 2012, M.E. Smith & D.H. Pfister DHP-CH-126, holotype SGO-169911; isotypes FLAS-F-62848 and FH-00290548.

Ascoma clavate, tapering at base, 60 mm high, 80 mm wide at the widest point, fleshy, forked and irregular, composed of several convoluted branches, each branch clavate to subcylindrical and resembling an individual ascoma of *Underwoodia singeri*, hollow and convoluted, partially emergent from the leaf litter. Hymenium adnate, smooth, slightly viscid, dark brown, covering the top half of the ascoma. Sterile base 30 × 20 mm, hollow inside, creamy tan, covering the lower half of the ascoma. Outer excipulum a palisade-like layer 180–240 µm, perpendicular to the outer surface, composed of isodiametric cells 7–14 µm diam, weakly cyanophilic. Subhymenium 360–400 µm thick, composed of prosenchymatous hyphae parallel to the surface. *Paraphyses* 8–12 µm wide, clavate, irregularly septate, obtuse at the apex, hyaline, equal to or exceeding the asci 10–20 µm. *Asci* (220–)240–320(–328) × 16–20 µm, cylindrical to clavate, usually 8-spored, dextrinoid when young, but nonreactive to Melzer's reagent when mature. *Ascospores* 20–27 × 10–11 µm, av. = 24.33 × 9.87 µm, Q = 2.5, biseriate when young but becoming uniseriate at maturity, ellipsoid, hyaline, containing a large central guttule, ornamented with low warts 1–2 µm high.

Habit, Habitat & Distribution — Beneath leaf litter but partially emergent, fruiting directly on soil; known from a single collection discovered in an old growth *Nothofagus dombeyi* forest in the Aysén region of Chile.

Notes — *Geomorium furciae* is easily separated from the other described South American species by a combination of the ascocarp morphology and spore dimensions. The ascocarp shape is clavate to convoluted in *G. furciae* whereas all other South American species are either truffle-like (*G. gamundiae*), columnar (*G. fuegianum*), or elongated and tooth-like (*G. singeri* and *G. geodon*). The ascospores of *G. furciae* are similar to those of *G. singeri*, *G. geodon*, and *G. fuegianum*, but are longer and more ellipsoid. Phylogenetically, this taxon is highly divergent and sister to all other known taxa in the *Geomoriaceae* (Fig. 1, 3).

Geomorium gamundiae Kraisit., Pfister, Mujic, Healy & M.E. Sm., *sp. nov.* — MycoBank MB828331; Fig. 6

Etymology. Named in honour of Dr. Irma Gamundí in recognition of her lifetime contribution to the study of discomycetes and Patagonian fungi.

Typus. ARGENTINA, Río Negro, Nahuel Huapi National Park, Los Rapiños near Brazo Tronador, in a mature *Nothofagus dombeyi* forest, on soil, 18 Mar. 2012, M.E. Smith & D.H. Pfister MES-577, holotype CORD-C00006469; isotypes FLAS-F-62851, FH.

Ascomata globose to irregularly lobed, highly convoluted, white throughout when young, becoming yellowish to light brown

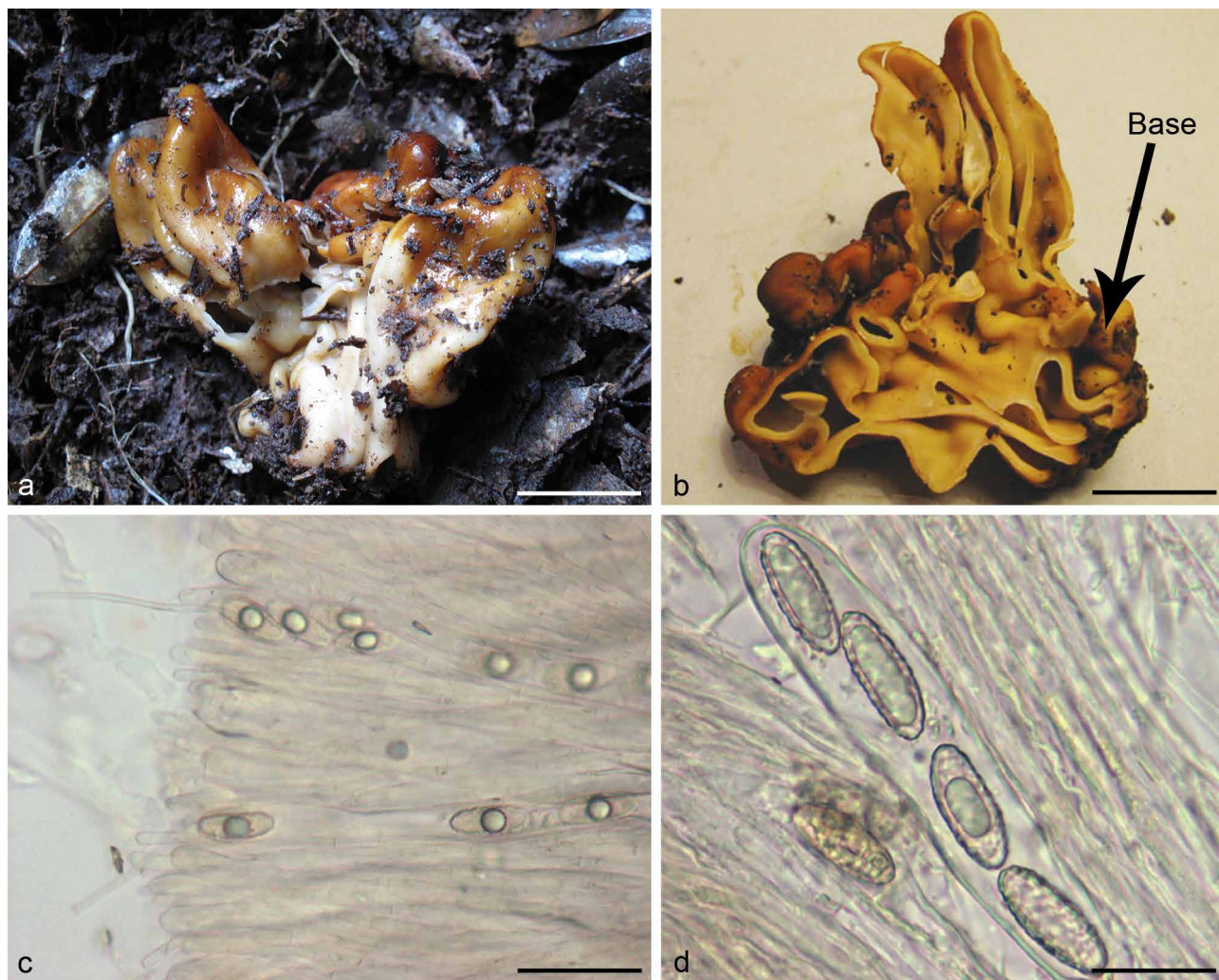


Fig. 5 Morphology of *Geomorium furciae*. a. An ascoma in the field (DHP-CH-126); b. cross section of an ascoma showing hollow cavities inside; c. hymenium showing paraphyses that equal or slightly exceed asci in length; d. mature ascospores showing large central guttules and ornamentation of low warts. — Scale bars: a–b = 2 cm; c = 30 µm; d = 20 µm.

in age or when dry, 10–25 mm diam, firm when fresh, with the odour of garlic or parmesan cheese. Gleba with hollow chambers formed by the in-folding of excipulum and hymenium. Hymenium composed of cylindrical asci and paraphyses pointing inwards forming a palisade, but with no obvious epithecium, often with soil particles adhering to the surface. Paraphyses irregularly septate, irregularly branched at base, swollen up to 14 µm wide at or near the apices, exceeding the asci by 20–40(–48) µm. Asci cylindrical, 280–340 × 16–24 µm, rounded at the apex, tapering towards the base, mostly 8-spored, sometimes with one or two aborted ascospores visible in the ascus, hyaline in water and 5 % KOH, contents of immature asci dextrinoid in Melzer's reagent but no reaction in mature asci. Ascospores biseriate when young, uniseriate or sometimes irregularly biseriate when mature, globose to subglobose, 12–16 µm diam, av. = 13.48 µm, Q = 1.0, excluding ornamentation, hyaline to pale yellow at maturity, ornamented with crowded, irregular warts 4–6 µm high and 2–4 µm wide.

Habit, Habitat & Distribution — Ascomata solitary or in groups, hypogeous in soil and leaf litter in *Nothofagaceae*-dominated forests. Known only from Nahuel Huapi National Park in Argentina but likely more widespread in *Nothofagaceae* forests in both Chile and Argentina. Confirmed as an ectomycorrhizal fungus associated with *Nothofagaceae* (Fig. 1, 2).

Additional specimens examined. ARGENTINA, Río Negro, Nahuel Huapi National Park, Arroyo Goye, in forest of *Nothofagus dombeyi* near site of invasion by *Pseudotsuga menziesii* (41° 6' 25.3S – 71° 31' 14.3W), 926 m above

sea level, on top of deep soil beneath extensive deposits of organic matter, by a small sink at the edge of trail, 26 Apr. 2017, A.B. Mujic AM-AR17-028 (CORD-C00006470, FLAS-F-62850); San Carlos de Bariloche, Cerro Otto, under *Nothofagus pumilio*, 800 m above sea level, 22 Apr. 2001, M. Castellan JT-26128 (OSC).

Notes — *Geomorium gamundiae* is the only fully hypogeous and truffle-like species of *Geomorium* from South America and is morphologically distinct from any known taxon in this group. The overall morphology of *G. gamundiae* is similar to the Australian species *G. australiana* and *G. echinulata* except that both of these taxa have more ellipsoid spores and reticulate to echinate ornamentation. In contrast, *G. gamundiae* has ascospores that are notably warty and subglobose.

We also examined a specimen found by Dr Roland Thaxter near Concepción, Chile in 1906 that is morphologically similar to *G. gamundiae*. The specimen, '*R. Thaxter Concepción Hypogeous #3*' from the Farlow Herbarium at Harvard University (FH-00284257), consists of a few small pieces of tissue in a vial with an unknown liquid. The spore dimensions for this species are slightly different than *G. gamundiae* and the spore ornaments are notably smaller which suggests that Thaxter's collection may represent another, related species. The existence of this specimen suggests that future collecting in the northern ranges of *Nothofagaceae* in South America may reveal additional new truffle-like taxa.

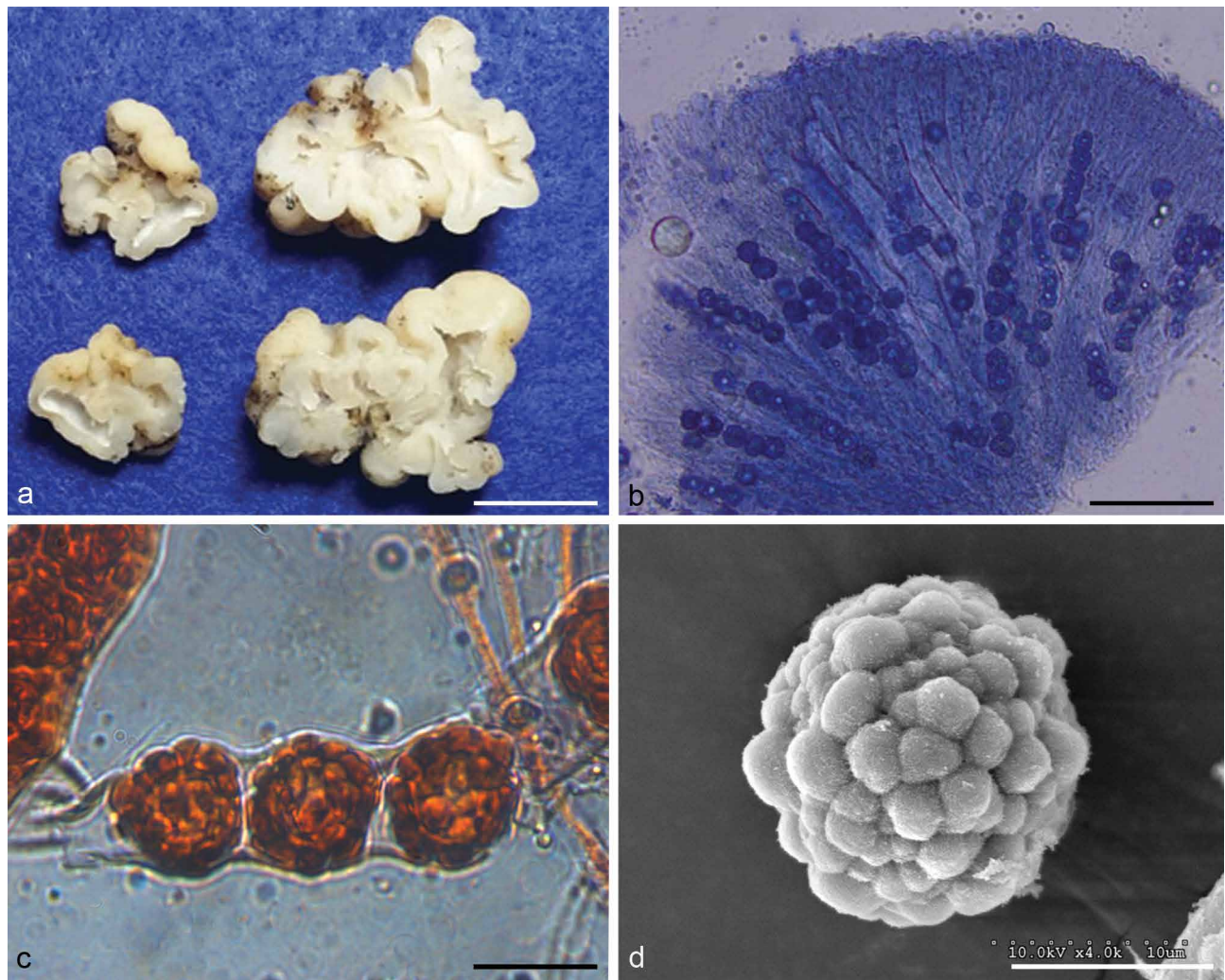


Fig. 6 Morphology of *Geomorium gamundiae* a. Fresh ascomata (AM-AR17-028) cut into cross sections showing the outer convoluted hymenium and the lack of peridium; b. section of an ascoma (MES-577) stained in cotton blue showing paraphyses that exceed the asci in length; c. light micrograph of mature ascospores stained in Congo red revealing the large warty spore ornamentation; d. mature ascospores viewed with the scanning electron microscope. — Scale bars: a = 1 cm; b = 50 μ m; c–d = 10 μ m.

Geomorium geodon Kraisit., Pfister, Mujic, Kuhar & M.E. Sm.,
sp. nov. — MycoBank MB829507; Fig. 7

Etymology. The epithet ‘*geodon*’ is derived from Greek ‘geo-’ referring to earth, and Greek ‘-odon’ referring to tooth.

Typus. CHILE, Magallanes, Magallanes National Park, mirador Las Minas, (53 8 21.5 S–71 3 29.3 W), 416 m above sea level, in deep soil along gentle slope in a *Nothofagus pumilio* forest, 3 Apr. 2017, A.B. Mujic MES-2362, holotype SGO-169913; isotype FLAS-F-62852.

Ascomata 40–100 $\times$ 8–15(–33) mm, cylindrical to occasionally claviform, slightly curved, acute to obtuse at the apex, fragile, hollow inside. Stipe occupying the lower half of the ascoma, smooth to longitudinally plicate, creamy white. Outer excipulum a palisade-like layer, 180–240 μ m, perpendicular to the outer surface, composed of isodiametric cells 7–14 μ m diam, strongly cyanophilic. Hymenium covering the top half to third of the ascoma, texture smooth, ranging from dry to viscid when fresh, dark purple to purplish brown when fresh but becoming successively browner when dried. **Paraphyses** simple, septate, 7.7–11.5 μ m wide at the apex, with dense brown to ochraceous pigment in the apical cells, even with or exceeding the asci by 16–50 μ m. **Asci** typically with eight ascospores, non-reactive in Melzer’s reagent, (200–)240–375 $\times$ (16–)18–20 μ m. **Ascospores** uniseriate, ellipsoid, 24–26 $\times$ 11–12.5 μ m, av. = 25.23 $\times$ 11.79 μ m, Q = 2.14, densely ornamented with warts 1.5–3 μ m high, hyaline to light yellow, typically containing one central guttule but sometimes with two or three guttules.

Habit, Habitat & Distribution — Single or occasionally in clusters, fruiting directly from soil among leaf litter. Found in *Nothofagaceae* forests in both Chile and Argentina.

Additional specimens examined. ARGENTINA, Neuquén, Nahuel Huapi National Park, Ultima Esperanza/Lago Espejo Trail, near Villa La Angostura, on soil, 13 May 2015, M.E. Smith MES-1239 (CORD-C00006473). — CHILE, Magallanes, Magallanes Forest Reserve, group camping site B (53 8 34.9S – 71 1 50.9W), 393 m above sea level, in a *Nothofagus pumilio* forest by the stream, in soil directly emerging from litter, 3 Apr. 2017, M.E. Smith MES-2377 (FLAS-F-62853, SGO-169912).

Notes — Unfortunately, we were unable to locate the type specimen of *Underwoodia singeri* var. *fulvostipitata*. Our collections listed here roughly match the description of this variety (Gamundí & Horak 1979) so we suspect that our new taxon corresponds to this variety. In order to avoid future confusion, however, we describe this species based on new and phylogenetically characterized specimens. We name this species *Geomorium geodon* in reference to its appearance as a large tusk or tooth emerging from the soil and also in reference to the informal name that was used by Roland Thaxter. *Geomorium geodon* is morphologically similar to *G. singeri* and the two species can be easily confused. *Geomorium geodon* tends to have a more purplish hymenium as compared to browner tones in *G. singeri* but this character may be difficult to distinguish in the field and colours are variable in both species. Microscopically, the ascospores of *G. geodon* are longer on average than those of *G. singeri* and the paraphyses of *G. geodon* contain

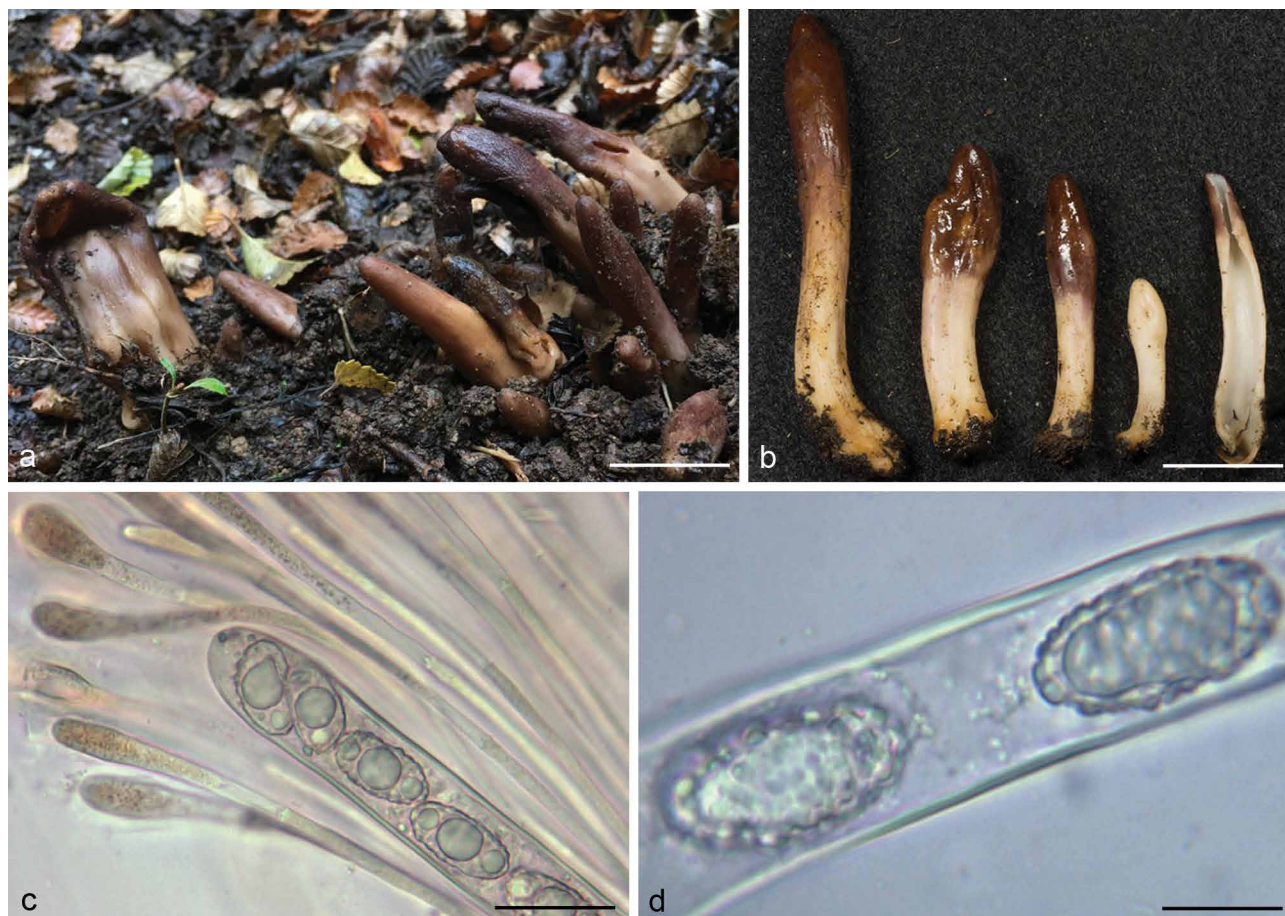


Fig. 7 Morphology of *Geomorium geodon* a. Aggregated ascomata in the field as they emerge from the soil (MES-2377); b. fresh ascomata (MES-2362) showing brown with purple hue and slightly viscid apices. Note the immature ascoma (second from the righthand side) which lacks the dark purplish colour on the hymenium; c. paraphyses with clavate apices and dark brown pigments surrounding an ascus (MES-2362); d. mature ascospores in an ascus (MES-2362) showing a large central guttule and ornamentation of warts of varying sizes. — Scale bars: a–b = 3 cm; c = 30 μ m; d = 10 μ m.

conspicuous dark brown pigments (Fig. 7c) whereas the paraphyses in *G. singeri* are usually lighter with diffuse light yellow pigments (Fig. 4b). Our phylogenies also clearly separate these two species (Fig. 1–3). Based on our collections it appears that *G. singeri* is more common than *G. geodon* but this could be a result of the timing of our sampling and locations that we visited.

Geomorium singeri (Gamundí & E. Horak) Kraisit., Pfister, Kuhar & M.E. Sm., *comb. nov.* — MycoBank MB828306; Fig. 4a, b

Basionym. *Underwoodia singeri* Gamundí & E. Horak, Beih. Sydowia 8: 162. 1979.

Typus. ARGENTINA, Tierra del Fuego, Depto. Ushuaia, Lapataia, on soil, under *Nothofagus pumilio*, 14 Mar. 1975, leg. A. Gaiotti, holotype, LPS-38598, examined by F. Kuhar.

Ascomata (32–)60–140 $\times$ 4(–7) mm, buried 15–25 mm in the ground, typically cylindrical or tooth-like but sometimes clavate, straight to curved, acute to obtuse at the apex resembling the shape of an asparagus shoot, fragile, fleshy but tough upon drying, hollow. Stipe 30–115 mm in length, smooth to longitudinally sulcate, glabrous on the upper part but fibrillose at the base, typically creamy white but sometimes with brown or purple tones. Outer excipulum lacking hairs, a palisade-like layer 225–350 μ m thick, composed of cells 20–40 $\times$ 7–19.5 μ m, cyanophilic, perpendicular to the outer surface. Hymenium covering the upper half to third of the ascoma, light chestnut brown but sometimes with purple to olive tones, texture smooth, ranging from dry to viscid when fresh but becoming plicate

when dried. **Paraphyses** simple, capitate, often knobbed and irregularly swollen, with diffuse yellow pigments mostly at the apex, 7–9.6 μ m wide, equal to or exceeding the asci by 10–40 μ m. **Asci** 350–410 $\times$ 14–18 μ m, cylindrical, usually with eight spores, dextrinoid in Melzer's reagent when young but nonreactive when mature. **Ascospores** 21–26 $\times$ 9.6–13 μ m, av. = 21.44 $\times$ 10.97 μ m, Q = 1.95, uniseriate, ellipsoid somewhat acute at the poles, ornamented with irregularly rounded warts 1.5–2.4 $\times$ 0.5–1.2 μ m, hyaline to pale yellow, containing one large central guttule or a few small guttules.

Habit, Habitat & Distribution — Single or occasionally in clusters, fruiting directly from soil among leaf litter in association with host trees in the *Nothofagaceae*. Found in both Chile and Argentina.

Additional specimens examined. ARGENTINA, Río Negro, Bariloche, Nahuel Huapi National Park, road to Tronador, after Pampa Linda, before bridge, 926 m above sea level, near *Nothofagus dombeyi*, on soil, 14 May 2016, A.B. Mujic MES-1986 (CORD-C00006472). — CHILE, Karukinka Reserve, around Vicuña station (54 08.31S – 68 42.68W), 172 m above sea level, in *Nothofagus pumilio* forest, close to the edge with *N. antarctica*, open and grazed area with dense understory, on soil, 26 Mar. 2017, T. Niskanen & C. Truong CT-4611 (FLAS-F-62902); Los Lagos, Vicente Perez Rosales National Park, on the road to the ski area above Mirador el Bosque, 930 m above sea level, in *Nothofagus dombeyi* forest, on soil, 17 Apr. 2017, M.E. Smith MES-2917 (FLAS-F-63313, SGO-169915); Los Lagos, Puyehue National Park, below Antillanca on the edge of the road, c. 1000 m above sea level, in deep Chusquea patches with *Nothofagus dombeyi* trees, on soil, 6 May 2015, D.H. Pfister & M.E. Smith MES-1073 (FLAS-F-63075); Los Lagos, Puyehue National Park, big flat area with arroyo below Antillanca, in *Nothofagus pumilio* forest, on soil, 12 Apr. 2017, M.E. Smith MES-2766 (SGO); Magallenes, Punta Arenas, [Las Minas], 1 Mar. 1906 (FH-00640352, FH-00640353); Magallenes, Río Santa María, just south of Reserva San Juan

and Fuerte Bulnes (53 40 27.7S – 70 59 21.6W), 17 m above sea level, in a forest dominated by *Nothofagus betuloides* with some *Nothofagus pumilio*, on soil, 1 Apr. 2017, M.E. Smith MES-2297 (SGO-169919); Magallanes Forest Reserve, Summit Area, 425 m above sea level, on soil, 21 Mar. 2008, M.E. Smith MES-155 (FH-00940350), MES-156 (FH-00940349), MES-161 (FH-00940351); Magallanes Forest Reserve, group camping site B (53 8 34.9S – 71 1 50.9W), 393 m above sea level, with *Nothofagus pumilio* along edge of depression between hillocks, on soil, 3 Apr. 2017, A.B. Mujic MES-2390 (FLAS-F-63307, SGO-169917); Magallanes Forest Reserve, on Sendero de Chile across the street from the parking lot above the park ranger's station (53 8 38.9S – 71 0 12W), 349 m above sea level, in *Nothofagus pumilio* forest with *N. betuloides* at forest edges, on soil, 4 Apr. 2017, M.E. Smith MES-2440 (FLAS-F-63309, SGO-169916); Magallanes Forest Reserve, near the park ranger station (53 8 34.3S – 71 0 21.9W), 341 m above sea level, in a *Nothofagus pumilio* forest, on soil, 7 Apr. 2017, M.E. Smith MES-2572 (FLAS-F-62861, SGO-169918); Punta Arenas, Club Andino ski area entrance (53 9 35.2S – 71 1 24W), 384 m above sea level, near *Nothofagus betuloides* and *N. pumilio*, on soil, in deep litter, 31 Mar. 2017, M.E. Smith MES-2266 (SGO-169914); Magallanes Forest Reserve, at the overlook, Río Las Minas (53 7 49.99S – 71 0 32.04W), 19 Mar. 2008, D.H. Pfister & M.E. Smith DHP-CH-20, (FH-00284813).

Notes — The description of *G. singeri* is taken primarily from the original description of *Underwoodia singeri* (Gamundí & Horak 1979) with slight modifications based on our morphological analysis of numerous collections, including the type specimen. Our morphological and phylogenetic analyses (Fig. 1–3) indicate that there are two distinct species within *Underwoodia singeri* s.lat. and that these likely correspond to the two described varieties, var. *singeri* and var. *fulvostipitata*. We examined the type of *U. singeri* and refer this to *G. singeri* s.str. However, we have been unable to locate the type specimen of *U. singeri* var. *fulvostipitata* to determine if it corresponds to the second species as we suspect. In order to avoid future nomenclatural and taxonomic confusion we describe a new species (see *G. geodon*). It seems likely that *G. geodon* corresponds to *U. singeri* var. *fulvostipitata* but this remains unverifiable. Unbeknownst to Gamundí & Horak (1979), *G. singeri* s.lat. had previously been collected by Roland Thaxter during his visit to Patagonia in 1906. In his unpublished diaries at the Farlow Herbarium at Harvard University, Thaxter informally referred to his collections as 'Geodon, the earth tooth' because of their resemblance to a tooth or tusk emerging from the ground (Pfister & Smith, pers. comm.). It is unclear whether Thaxter's collections correspond to *G. singeri* s.str. or the morphologically similar species described above as *Geomorium geodon* because both species occur in the region near Punta Arenas (Chile), where Thaxter was collecting in 1906.

Excluded species

Gymnohydnotrya ellipsospora (J.W. Cribb) B.C. Zhang & Minter, Mycol. Res. 92(2): 196. 1989

Basionym. *Sphaerozone ellipsosporum* J.W. Cribb, Paper of the Department of Botany, University of Queensland 4: 36. 1960.

Notes — We have neither examined any specimens of *Gymnohydnotrya ellipsospora* nor are there any molecular data available for this taxon. Beaton & Weste (1978) stated that the holotype specimen (at BRIU) is lost and the isotype (at OSC) is in poor condition. The original description lacks a morphological analysis and there are no good illustrations of excipulum, asci or paraphyses. With limited information, we cannot conclude with confidence that this taxon belongs to the genus *Geomorium*. It is probable that this is a synonym of *G. echinulatum* or a closely related species due to morphological similarities. Freshly collected samples are needed for morphological and molecular analyses to resolve its phylogenetic position.

DISCUSSION

This is the first comprehensive systematic study of the species previously treated in the genera *Underwoodia* and *Gymnohydnotrya*, the /gymnohydnotrya lineage of Bonito et al. (2013). Our multi-locus phylogenetic analysis indicates that this clade is highly supported and not nested in any of the described families in the *Pezizales* (Fig. 1). We resurrect the genus *Geomorium* to apply to these Southern Hemisphere taxa because the type of the genus *Underwoodia*, known from North America, is distantly related and belongs to *Helvellaceae*.

Our analysis placed *U. columnaris* as sister to the rest of the *Helvellaceae* (e.g., *Balsamia* and *Helvella* spp.), as was shown previously in the 18S and 28S analyses of O'Donnell et al. (1997). Their molecular data also included species of *Barssia* and *Wynnella*. The 28S analysis of Læssøe & Hansen (2007) also placed *U. columnaris* within the *Helvellaceae*, but without strong support for the relationships among genera in the family. We suggest that additional sequences of *rpb2* and *EF1a* from *Barssia* and *Wynnella* species may help to further refine the placement of *U. columnaris*.

The generic name *Gymnohydnotrya* exists for the Southern Hemisphere taxa studied here but *Geomorium* is the older name and therefore has precedent (Spegazzini 1922, Zhang & Minter 1989). The *Geomorium* species are morphologically, biogeographically and phylogenetically distinct from taxa in the *Helvellaceae* or *Tuberaceae*. Although it is phylogenetically acceptable to include the genus *Geomorium* in *Tuberaceae*, we chose to erect the new family *Geomoriaceae* to accommodate these fungi for several reasons. First, the vast majority of *Tuberaceae* have enclosed truffle-like ascomata with a solid or semi-solid gleba and distinct peridia (stereothecia) (Bonito et al. 2013), whereas most members of the *Geomoriaceae* have either columnar, clavate, tooth-like (apothecia) or truffle-like ascomata without peridia (exothecia). Second, most taxa in the *Tuberaceae* have globose to subglobose asci with globose ascospores while all members of *Geomoriaceae* have cylindrical asci and most have ellipsoid ascospores. The newly described species *G. gamundiae*, with globose to subglobose ascospores, is an exception to this rule. Finally, it is significant to the justification for the family that all taxa thus far known are from the Southern Hemisphere and are ectomycorrhizal with austral plants, indicating a deep co-association. It is likely that more taxa in this family are awaiting discovery in *Nothofagaceae* forests in Australasia and South America. More sampling is thus essential to assess the full diversity of *Geomoriaceae*.

Our phylogenetic results also infer that the *Tuberaceae*, *Helvellaceae*, and *Geomoriaceae* together form a clade (Fig. 1, 3). It is notable that most members of *Helvellaceae* and *Geomoriaceae* have epigeous ascomata that are columnar or stipitate with a modified apothecium, e.g., cupulate or saddle-shaped. The shared morphology between these two groups along with the apothecial species *Nothofajnea thaxteri* (*Tuberaceae*) suggests that the most recent common ancestor of the entire lineage likely produced apothecia or modified apothecia. Furthermore, the distribution of hypogeous ascomata across several lineages in *Tuberaceae*, *Helvellaceae*, and *Geomoriaceae* suggests that the truffle-like morphology evolved several times independently within these three families. A similar pattern of truffle evolution has previously been observed in *Helvellaceae* and *Tuberaceae* as well as in other groups of *EcM Pezizales* (O'Donnell et al. 1997, Læssøe & Hansen 2007).

Our divergence time estimation suggests that *Geomoriaceae* arose sometime between 112–67 MYA (Fig. 3), which coincides with the origin of *Nothofagaceae* in the Southern Hemisphere (Knapp et al. 2005). In contrast, divergence time estimation

using secondary fossil calibration (O'Donnell et al. 2011) suggests that *Geomoriaceae* arose at least 40 MY earlier (Fig. S1). However, this date precedes the estimated emergence of both *Nothofagaceae* and *Myrtaceae* ECM tree hosts in the late Cretaceous (Sanmartín & Ronquist 2004, Sytsma et al. 2004). Evidence reveals that all *Geomoriaceae* share the ECM trophic mode (Fig. 1, 2), and thus suggests that the divergence time estimation based on the rate of 28S rDNA molecular evolution (Bonito et al. 2013) is a more robust hypothesis (Fig. 3).

In the ITS and the multi-locus analysis, all of the represented Australasian species in the *Geomoriaceae*, i.e., *G. australianum*, *G. beatonii*, and *G. echinulatum*, formed a clade, albeit without strong statistical support (Fig. 1, 2). In our dating analysis, *G. australianum* was inferred as sister to *G. singeri*, but also with no support (Fig. 3). Divergence time estimation suggests that the Australasian lineages separated from their South American sister lineages during the mid-Paleogene period, c. 50–40 MYA (Fig. 3). This evolutionary event coincides with the split of South America from Australasia and Antarctica (Sanmartín & Ronquist 2004). It has been hypothesized that the break-up of Southern Gondwana (Antarctica, Australia, South America) facilitated vicariant diversification in several groups of animals and plants (Sanmartín & Ronquist 2004). For instance, a molecular clock analysis shows that the genus *Lophozonia* (*Nothofagaceae*) diverged into South American and Australasian clades around the time of the Gondwanan break-up (Knapp et al. 2005). A similar biogeographic divergence pattern has been observed in several other lineages of fungi in the Ascomycota such as in the genera *Aleurina* (Tedesoo & Smith 2013), *Cyttaria* (Peterson et al. 2010), and *Ruhlandiella* (Kraisitudomsook et al. 2019). We postulate that the Gondwanan separation caused vicariant events within the *Geomoriaceae* as well. More Australasian samples are needed to obtain a more complete evolutionary history of *Geomoriaceae* and to further document these austral fungal distributions.

KEY TO DESCRIBED SPECIES OF GEOMORIUM

1. Ascomata found in South America, near *Nothofagus*, *Lophozonia*, or other ectomycorrhizal *Nothofagaceae* 2
1. Ascomata found in Australasia near *Eucalyptus*, *Melaleuca*, or other ectomycorrhizal *Myrtaceae* 3
2. Ascomata hypogeous, subglobose-globose, highly convoluted, hollow inside *G. gamundiae*
2. Ascomata modified columnar apothecium, epigeous to semi-hypogeous 5
3. Ascomata columnar, morel-like, epigeous, apex slightly curved, horn-like *G. beatonii*
3. Not as above, ascomata subglobose to globose, hypogeous, convoluted 4
4. Ascomata each with many cavities, hymenium present both externally and internally, ascospores slightly ornamented, reticulate *G. australianum*
4. Ascomata each with one cavity, hymenium present internally, ascospores ornamented conspicuously with spines up to 4.5 µm long *G. echinulatum*
5. Hymenium layer viscid, brown, semi-hypogeous, ascoma hollow and composed of several convoluted, wide, column-like branches, rare and known only from coastal *Nothofagaceae* forests *G. furciae*
5. Not as above, ascomata epigeous and more regular or columnar in shape 6
6. Ascomata columnar 10–30 mm wide, ridged and wrinkled on the outside, hollow with multiple narrow chambers, white at the bases but black at the apices *G. fuegianum*

6. Not as above, ascomata 3–15 mm wide, mostly smooth on the outside, hollow with few or no chambers, resembling the shape of an asparagus shoot 7
7. Ascospore ellipsoid with an average of 21 µm long and Q-ratio of 1.95, paraphyses with diffuse yellow pigments at the apex *G. singeri*
7. Ascospore ellipsoid with an average of 25 µm long and Q-ratio of 2.14, paraphyses with dense brown to ochraceous pigments at the apex *G. geodon*

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Supplementary material

Fig. S1 Chronogram estimation of *Geomoriaceae* and related species obtained from BEAST analysis of three concatenated genes (28S rDNA, *rpb2*, *EF1 α*) with a log-normal distributed clock model and two secondary calibration points based on O'Donnell et al. (2011). Number above the nodes represents the mean estimated divergence time with a black bar representing the 95 % confidence interval. All nodes receive maximum Bayesian Posterior Probability unless noted below the node. Sequences of type specimens are highlighted in **bold**. Specimen voucher numbers are indicated after species names.